Effect of extra cysteine residue of new mutant 1Ax1 subunit on the functional properties of common wheat

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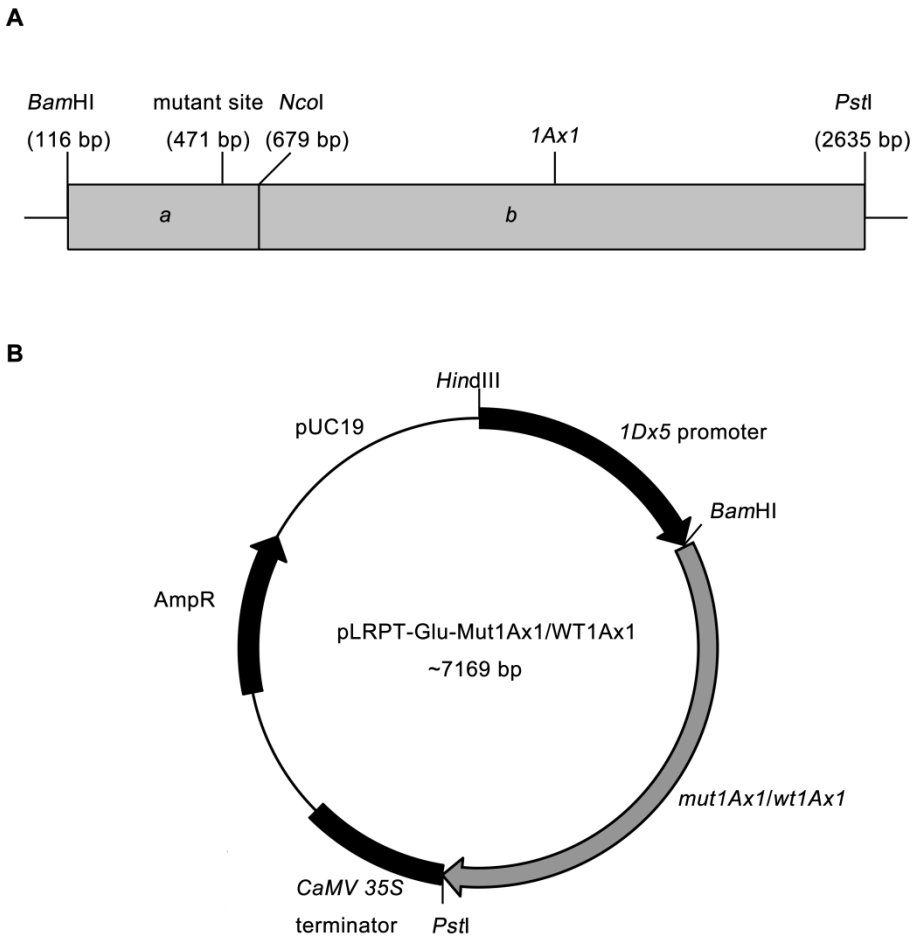

Figure S1. Schematic diagrams of the *mut1Ax1* gene and plasmid pLRPT-Glu-Mut1Ax1/WT1Ax1 with the position of relevant restriction sites. (**A**) The *mut1Ax1* gene was generated from a combination with the one part (*a*) obtained from chemical synthesis and another part (*b*) cut from the plasmid pHMW1Ax1. (**B**) The *mut1Ax1* or *wt1Ax1* gene was inserted between the endosperm-specific *1Dx5* promoter and the *CaMV35S* terminator.

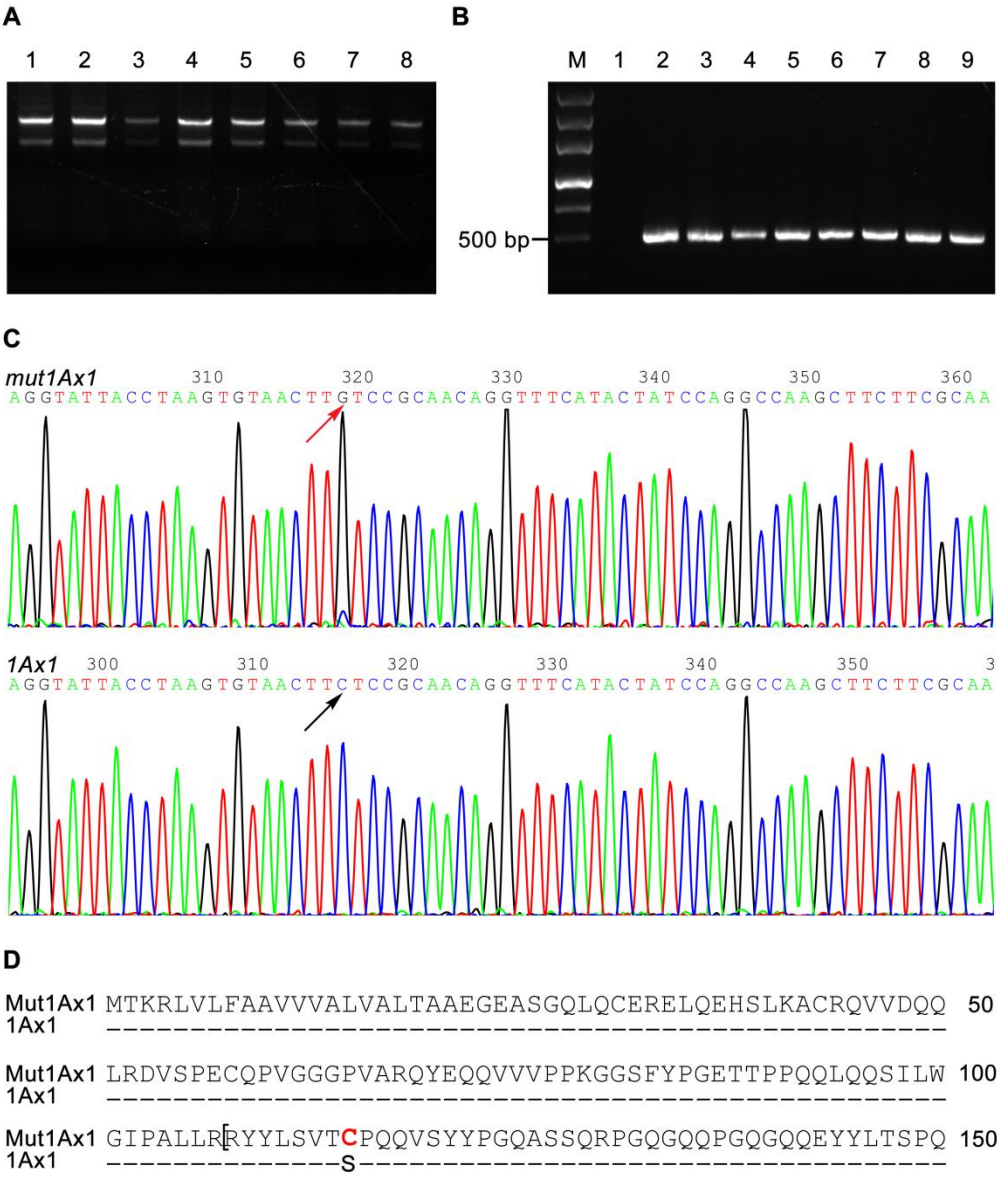

Figure S2. RT-PCR analysis of transgenic lines expressing *mut1Ax1* and *wt1Ax1* and partial comparison of the derived amino acid sequence of Mut1Ax1 with 1Ax1. (**A**) Identification for the integrity of mRNA extracted from seeds (15 dpa) of T_1 transgenic lines. (**B**) RT-PCR and (**C**) sequencing analyses of transgenic lines. (**D**) Partial comparison of the derived amino acid sequence of Mut1Ax1 with 1Ax1. The mutant site is indicated by the red arrow (**C**) and the red box (**D**).

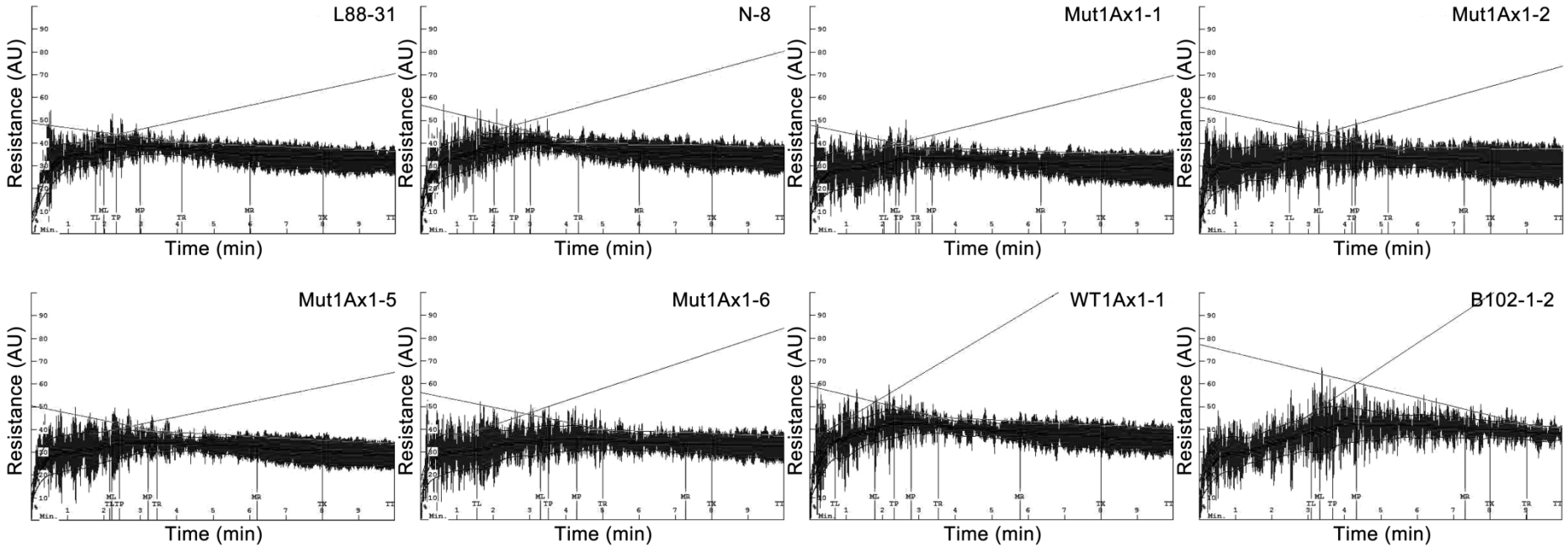

Figure S3. Dough sample mixing curves of transgenic and control wheat lines.

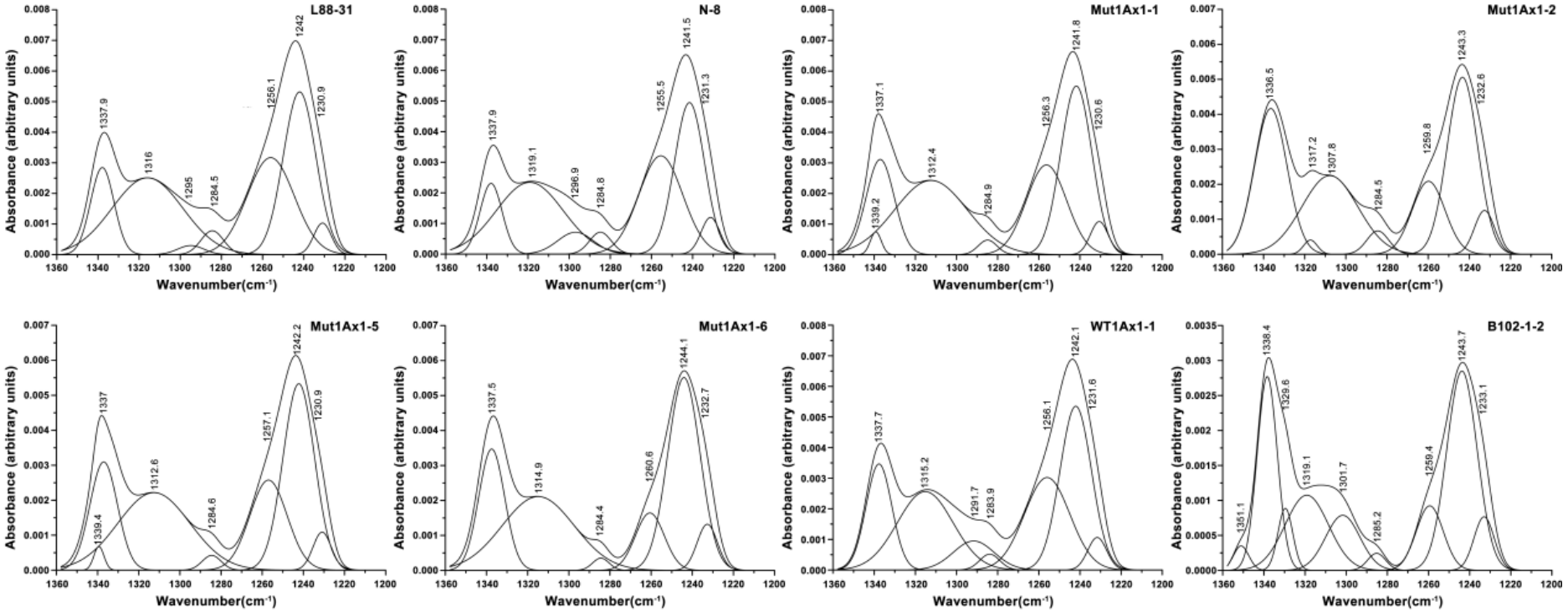

Figure S4. FT-IR spectra in the 1,360 – 1,200 cm^{-1} range for dough samples of the transgenic and control wheat lines.

Parameters	**Lines**							
	L88-31	**N-8**	**Mut1Ax1-1**	**Mut1Ax1-2**	**Mut1Ax1-5**	**Mut1Ax1-6**	**WT1Ax1-1**	**B102-1-2**
Plant height (cm)	101.8abABC	96.4cC	103.2abABC	102.8abABC	107.1aA	100.5bcABC	104.6abAB	99.5bcBC
Heading date (days)	161.2	163.4	163.4	161.7	162.4	163.5	163.8	161.4
Growth period (days)	210.5abA	211.8aA	206.8bcAB	210.3abA	207bcAB	209.8abA	212aA	204cB
No. of spikelets per spike	18.8	20.4	19.9	19.4	20.4	19.8	20.1	19.8
No. of seeds per spike	42.85bB	44.1bB	52.1aAB	43.8bB	53.3aAB	56.6aA	49.4abAB	43.6bB
1,000-seed weight (g)	26.8	27.7	27.0	26.8	27.8	27.6	27.2	27.7

Table S1 Agronomic performance of transgenic and control wheat lines. Values within the same parameter followed by the same letter are not significantly different at the 0.05 (small letter) and 0.01 (capital letter) probability levels. Each average was calculated from fifteen biological experiments.

Parameters	**Lines**							
	L88-31	**N-8**	**Mut1Ax1-1**	**Mut1Ax1-2**	**Mut1Ax1-5**	**Mut1Ax1-6**	**WT1Ax1-1**	**B102-1-2**
Endogenous HMW	**17+18**	**17+18**	**17+18**	**17+18**	**17+18**	**17+18**	**17+18**	**17+18**
Transgenic HMW	NA	NA	Mut1Ax1	Mut1Ax1	Mut1Ax1	Mut1Ax1	1Ax1	1Ax1
Protein characterisation								
Flour protein content (%)[b]	7.52	7.89	8.27	8.21	8.21	8.72	8.46	9.29
Glutenin (μg mg^{-1} flour)[c]	3.55cC	3.53cC	3.82bcBC	4.01abAB	3.69cC	4.07aAB	3.62cC	4.19aA
Gliadin (μg mg^{-1} flour)[c]	4.97	4.91	4.41	4.67	4.63	4.76	4.75	4.67
Glutenin/gliadin	0.74bBC	0.72bC	0.87aAB	0.86aAB	0.8abABC	0.86aAB	0.75bBC	0.9aA
Gluten index[d]	44.21eE	44.56eE	53.4cdCD	59.47bcBC	53.7cdCD	63.51abAB	50.21deDE	67.99aA
SDS sedimentation (ml)[e]	12.53eD	12.87eD	16.13cC	15.27cdC	16.1cC	14.97dC	17.6bB	32.37aA

Table S2 Comparisons of flour quality-related parameters of the transgenic and control wheat lines[a]. NA, not applicable. [a] Results are expressed based on 14% moisture. [b] Protein contents were determined by the Dumas method with an average of 2 replications. [c] Glutenins and gliadins were determined using the Bradford assay with an average of 4 replications. [d] Gluten index was determined using AACC method 38-12A with an average of 4 replications. [e] SDS-sedimentation volume was determined according to AACC international method 56-70.01 with an average of 4 replications. Values within the same parameter followed by the same letter are not significantly different at the 0.05 (small letter) and 0.01 (capital letter) probability levels.